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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07J 1/00, A61K 31/56, 31/565 // C07J 21/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 95/10527</b> <b>(43) International Publication Date:</b> 20 April 1995 (20.04.95)
<b>(21) International Application Number:</b> PCT/US94/11655 <b>(22) International Filing Date:</b> 13 October 1994 (13.10.94) <b>(30) Priority Data:</b> 08/135,739 13 October 1993 (13.10.93) US <b>(71) Applicant:</b> NEUROCRINE BIOSCIENCES, INC. [US/US]; Suite 317, 1020 Prospect Street, La Jolla, CA 92037 (US). <b>(72) Inventors:</b> CHRISTENSEN, B., G.; 48 Philhower Road, Lebanon, NJ 08833 (US). WEBB, Thomas, Roy; 2250 Colony Terrace, Encinitas, CA 92024 (US). <b>(74) Agents:</b> HERMANN, Karl, R. et al.; Seed and Berry, 6300 Columbia Center, 701 Fifth Avenue, Seattle, WA 98104- 7092 (US).		<b>(81) Designated States:</b> AM, AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LT, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> 3,17-DIHYDROXY-3,7,16 AND/OR 17-METHYL-ANDROST-5-ENE COMPOUNDS, DERIVATIVES THEREOF, AND THEIR USE  <b>(57) Abstract</b>  Novel di- and tri-methyl androst-5-ene-3,17-diols, having from 1 to 2 methyl substituents and optionally an hydroxyl group at the 7 position are provided. The compounds may be used prophylactically and therapeutically for activities associated with dehydroepiandrosterone.		

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5     **3,17-DIHYDROXY-3,7,16 AND/OR 17-METHYL-ANDROST-5-ENE  
COMPOUNDS, DERIVATIVES THEREOF, AND THEIR USE**

INTRODUCTION

10    Technical Field

The field of this invention concerns androstene steroids and their therapeutic uses.

Background

15     Dehydroepiandrosterone ("DHEA") is the most abundant steroid produced in man and for many years was considered as an intermediate in the synthesis of sex steroids. It has long been known that DHEA levels decline with progressive age, so that an individual in his 80's may produce only 10-20% of what he made in his second decade. DHEA appears to have broad physiological activity and has been referred to as  
20    a buffer hormone, mediating a wide variety of physiological responses, depending upon the state of the host. See Regelson *et al.*, *Ann. N.Y. Acad. Sci.* (1988) 521:260-273. DHEA and ester derivatives thereof have been reported as having immune enhancing effects, so as to protect the hosts from a variety of diseases, particularly viral diseases, as well as enhancing immune response, where an immunogen or vaccine is  
25    administered to a host. DHEA has also been reported to be effective as an anti-obesity and weight-losing agent. DHEA has also been reported to be effective in the treatment of autoimmune diseases. DHEA is also reported to be a potent inhibitor of mammalian glucose-6-phosphate dehydrogenase, which enzyme is rate controlling in the pentose phosphate shunt and a major source of extramitochondrial NADPH. There is also  
30    a suggestion that DHEA may find use in tumor inhibition.

Because of the pluripotency of DHEA, there has been extensive interest in studying the use of DHEA as a therapeutic, as well as finding derivatives of DHEA, which would have greater specificity. For the most part, derivatives have been associated with the esterification of the 3-hydroxy. However, other derivatives have  
35    also been reported, where halogen has been substituted at the 16 position, as well as numerous other groups at a variety of other positions. Despite the extensive research, these derivatives have not found commercial use. There is, therefore, substantial interest in finding compounds which may provide one or more of the physiological

effects observed with DHEA, where side effects may be minimized and potency achieved at a similar or enhanced level.

#### Relevant Literature

- 5 U.S. Patent Nos. 2,170,124 and 2,251,586 describe the preparation of androstene derivatives. U.S. Patent Nos. 2,845,381; 4,518,595; 4,628,052; 4,666,898; 4,701,450; 4,956,355; and 5,001,119; and foreign patents DE 3812595 and WO92/03925 describe a variety of uses of DHEA and derivatives thereof having biological activity and therapeutic applications.
- 10 Descriptions of the use of DHEA and derivatives thereof having physiological activity in the scientific literature may be found in Regelson *et al.*, *Ann. N.Y. Acad. Sci.* (1988) 521:260-273 (a review article); Loria *et al.*, *J. Med. Vir.* (1988) 26:301-314; Danenberg *et al.*, *Antimicrobial Agents in Chemotherapy* (1992) 36:2275-2279; Loria and Padgett, *Arch. Virol.* (1992) 127:103-115; and Araneo *et al.*, *J. Inf. Dis.* 15 (1993) 167:830-840, and references cited therein.

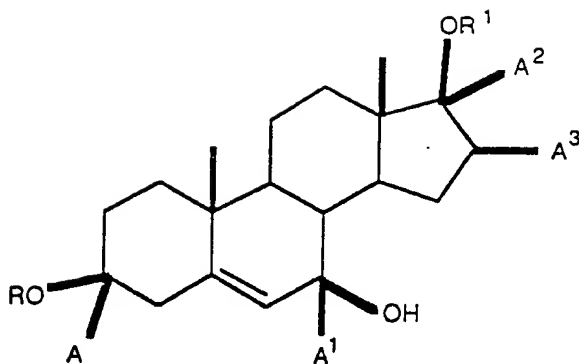
#### SUMMARY OF THE INVENTION

- 3, 7, 16 or 17 mono- and dimethyl substituted 3,17-dihydroxy-androstene-5, optionally substituted with hydroxyl at the 7 position and physiologically active esters and ethers thereof are provided. The compounds have broad biological activity *in vitro* 20 and *in vivo*, particularly enhancing the immune system, protecting against infection with pathogens, and in the treatment of a wide variety of physiological disorders.

#### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

- 25 In accordance with the subject invention, novel androstene-5 derivatives are provided, where the derivatives provide for a wide variety of physiological activities. Particularly, mono- and dimethyl substituted 3,17-dihydroxy-androstene-5 derivatives are provided, where the 1 to 2 methyl groups are at the 3, 7, 16 or 17 positions and 30 there is optionally an hydroxyl group at the 7 position. The hydroxyl groups are for the most part  $\beta$ -substituted, but may also be  $\alpha$ -substituted, particularly where a methyl group is substituted at the same position. The methyl groups may be  $\alpha$  or  $\beta$ , being primarily  $\alpha$  at other than the 16 position. The hydroxyl groups may be substituted with physiologically acceptable alkyl and acyl groups, particularly at the 3 and/or 17 35 positions. The acyl groups may be organic or inorganic.

For the most part, the compounds of this invention will have the following formula:



wherein:

the A groups are hydrogen or methyl, where not more than 2 of the A groups are methyl and wherein when dimethyl, combinations of particular interest include 3,7;  
 5 7,17; and 7,16;

the R groups are the same or different and are hydrogen, alkyl of from 1 to 6, usually 1 to 4, more usually 1 to 2 carbon atoms or a physiologically acceptable acyl of not more than about 12 carbon atoms, which includes sulfate, phosphate, phosphonate, and carboxylate, where the acid groups may be substituted with from 1 to 2 substituents  
 10 of from 1 to 12, more usually from 1 to 8, preferably from 1 to 6 carbon atoms, which may be aliphatic, alicyclic, aromatic or heterocyclic, where the various groups may be substituted with from 1 to 3 heterogroups, where the heteroatoms will be oxygen, nitrogen, halogen, usually chlorine or bromine, sulfur or the like.

Compounds of particularly interest based on androstene-5 include 3,7,17-tri- $\beta$ -  
 15 hydroxy-7- $\alpha$ -methyl; 3,7,17-tri- $\beta$ -hydroxy-17- $\alpha$ -methyl; 3,7,17-tri- $\beta$ -hydroxy-7,17-di- $\alpha$ -methyl; 3- $\alpha$ -hydroxy, 17- $\beta$ -hydroxy, 3- $\beta$ -methyl; 3,7,17-tri- $\beta$ -hydroxy-3,7-di- $\alpha$ -methyl; 3,17-di- $\beta$ -hydroxy-16- $\alpha$ - or  $\beta$ -methyl; 3,7,17-tri- $\beta$ -hydroxy-7- $\alpha$ -methyl-16- $\alpha$ - or  $\beta$ -methyl.

Esters of interest include physiologically acceptable sulfates and sulfatides (see  
 20 DE 3812595), phosphate, acetate, benzoate, oleate, and the like. The ester groups will, for the most part, vary the rate of metabolism, or enhance specificity as to particular tissues or cells.

Methods of preparation for the subject compounds are found in the literature, see for example, U.S. Patent No. 5,001,119 and particularly in the Experimental  
 25 section of the subject application. A wide variety of techniques may be used for introducing the hydroxyl and/or methyl groups at the various positions for the subject compounds and no particular method is considered critical to the subject invention. Groups at the 7 position can be achieved from the androst-5-ene-3,17-diol by oxidation at the 7 position, which can provide hydroxyl and methyl groups as appropriate.  
 30 Methyl groups may be introduced at the 17 position by employing the available ketone

and using a metal methyl derivative. Methyl groups may be introduced at the 16 position by using the 17-oxo derivative and treating the compound with a strong base to produce the 16-anion which may then be methylated with a methyl halide. Various techniques may be employed to obtain the desired isomer and stereoisomer, as  
5 appropriate.

The subject compounds may find application both *in vitro* and *in vivo*. The subject compositions may find use in inhibiting mammalian glucose-6-phosphate dehydrogenase (Oertal and Rebeun, *Biochem. Biophys. Acta.* (1969) 184:459-460). The subject compounds may also find use in prophylaxis and therapy associated with  
10 the immune system. Thus, the subject compounds may be used as adjuncts in conjunction with immunogens for production of antibodies or with vaccines for enhancing the immune response. For use as an adjuvant, the subject compounds may be administered prior to, concomitantly with or subsequent to the administration of the immunogen. The subject compounds, individually or collectively, will be at a  
15 concentration in the range of about 0.1 to 10 mg/kg. Various other components may be present in conventional amounts, such as BCG, alum lipids, e.g. muramyl phosphatides, mycolates, isoprinosines, etc. Conventional vehicles may be employed and when administered by injection will usually be administered intravascularly, intramuscularly, subcutaneously and the like. Booster administrations may be  
20 employed, which may be employed at intervals of from about 2 weeks to 1 year.

In addition, the subject compositions may be applied at the site of exposure to infectious organisms, e.g. during surgery, to prophylactic vaginal preparations, or as lubricants on condoms. For protecting against encephalitis and meningitis, the compositions may be administered intrathecally, either at the spinal level or into the  
25 cisterna magna. The subject compounds may be applied to the omentum in conditions such as endometritis and malignancies of the bowel and ovary. The subject compositions may be administered to enhance the immune response to pathogens, particularly viruses, more particularly retroviruses, e.g. lymphotropic viruses, such as HIV and HTLV, herpes viruses, enteroviruses, e.g. coxsackie virus, etc. The subject  
30 compounds may also be used in a prophylactic or therapeutic manner to protect against unicellular microorganisms, such as *Pseudomonas*, *Escherichia*, *Mycobacterium*, *Cryptosporidium* and *Streptococcus*.

The subject compositions may be administered when an individual patient is in an immunocompromised state for any reason, such as during infection, chemotherapy,  
35 cancer, or the like, so as to provide protection against invasion by opportunistic organisms or the occurrence of other diseases.

The subject compositions may also find application with autoimmune diseases. The subject compositions may be used in the treatment of such diseases as rheumatoid arthritis, osteoarthritis, lupus, diabetes and multiple sclerosis.

The subject compositions may be also used in the treatment of various cancers, particularly mammary cancer, ovarian cancer, lymphomas and leukemias.

The subject compositions also find application in their effect on lipid metabolism, where the subject compositions may be used for anti-obesity and weight-loss. The use of these compounds may be by themselves or in conjunction with other treatments, such as low cholesterol diets.

The subject compositions may also be employed in conjunction with thyroid dysfunction, since it is found during thyroid dysfunction, that DHEA levels are diminished and the ratio of DHEA to its sulfate ester are changed. Other applications for the subject compositions include as an antagonist to the production of TNF, IL-1 $\beta$ , IL-6, IL-8 and other pro-inflammatory cytokines.

The mode of administration of the subject compositions will vary depending upon the particular composition, the indication to be treated, the number of administrations, whether a single dose or repetitive doses, the activity of the compound based on the mode of administration, and the like. The compounds may be administered orally, parenterally, e.g. intravascularly, subcutaneously, intraperitoneally, intramuscularly, topically, etc., or by inhalation.

Depending upon the mode of administration, a wide variety of physiologically acceptable vehicles may be employed. Since the subject compositions are lipophilic, inert diluents will include lipids or aqueous dispersions. Alternatively, the active compound may be incorporated with excipients and prepared as various tablets, particles, capsules, or the like. For oral therapeutic administration, the active compound may be prepared as ingestible or buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers or the like. For parenteral administration, the subject compositions may be used in sterile solutions, such as saline, aqueous glucose, aqueous alkanol, or the like. For subcutaneous administration, the subject compositions may be used with alkanolic dimethylsulfoxide, or the like.

The liquid forms of the subject composition may include a wide variety of physiologically acceptable additives, such as surfactants, particularly non-ionic surfactants, such as hydroxypropyl cellulose, polyethylene glycols, etc. Media which may be employed include water, ethanol, polyols, e.g. glycerol, propylene glycol, etc., vegetable oils, lecithin, etc.

Other materials which may be present may be bactericides and anti-fungal agents, isotonic agents, sugar, and the like.

Formulations of steroids are conventional and find extensive exemplification in the literature. See for example, U.S. Patents 4,448,774; 5,043,165; 4,904,474; and 4,279,900.

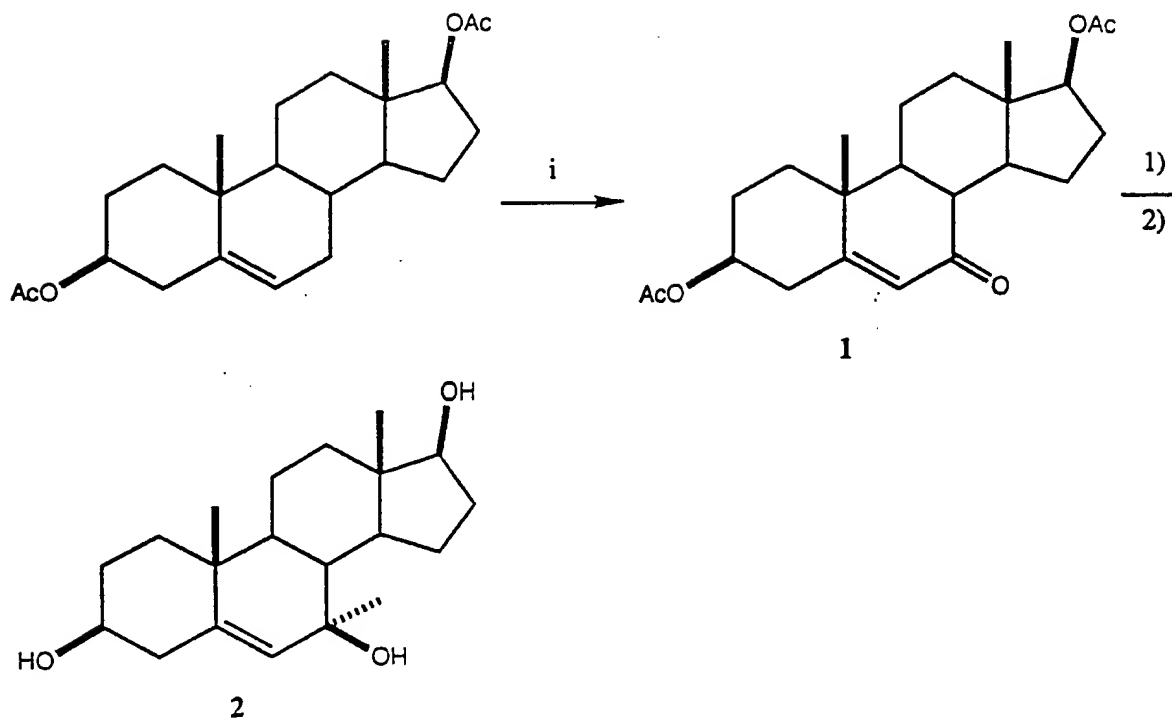
Depending upon the mode of administration, the dosage may be widely varied.

- 5 For all dosages, from about 100  $\mu$ g to 500 mg/kg/day may be employed. For parenteral administration the dosage may vary from about 0.1 to 50 mg/kg/day of host.

- As to each compound and indication, for the most part the dosage will be initially determined empirically based on efficacy and safety. The manner of determining safe dosage is well established, using animals initially, where animal  
10 subjects can provide safety, and where animal models are available, efficacy and predicted dosages for use in humans may be obtained.

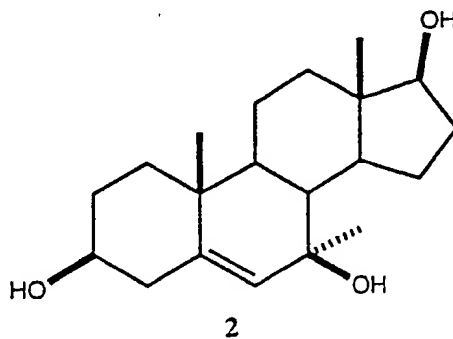
The following examples are offered by way of illustration and not by way of limitation.



EXPERIMENTAL

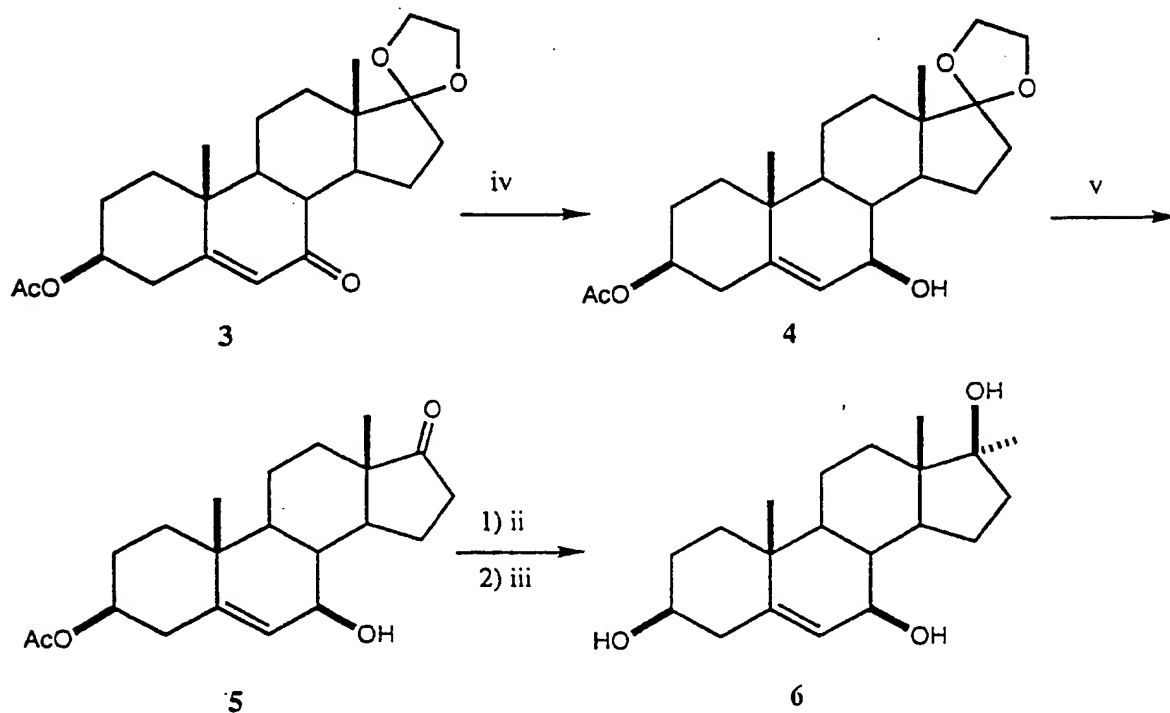
i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water

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**Example 1**10 Synthesis of 7 $\alpha$ -Methyl-3 $\beta$ , 7 $\beta$ , 17 $\beta$ -Trihydroxvandrost-5-ene(2):

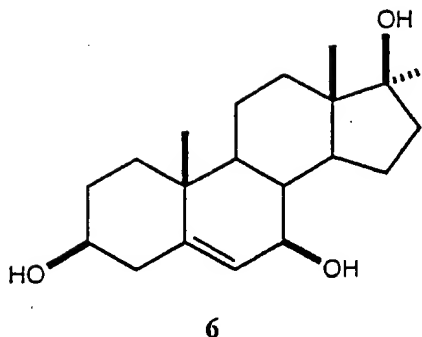
3 $\beta$ ,17 $\beta$ ,-Diacetoxyandrost-5-en-7-one (1) is prepared according to a known procedure (US Patent 5,206,008). A solution of 1 (499 mg, 1.28 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of  $\text{CH}_3\text{MgCl}$  (Aldrich) in THF. After the addition is > 90% complete (~15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction

mixture is then cooled in an ice-bath and treated with 50 ml of 1 M  $\text{NH}_4\text{Cl}$  in water over a 5 min. period. The resulting mixture is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give the pure title compound. The product is characterized by NMR, mass spectra, and elemental analysis.



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$   
v)  $\text{H}_3\text{O}^+$

## Example 2

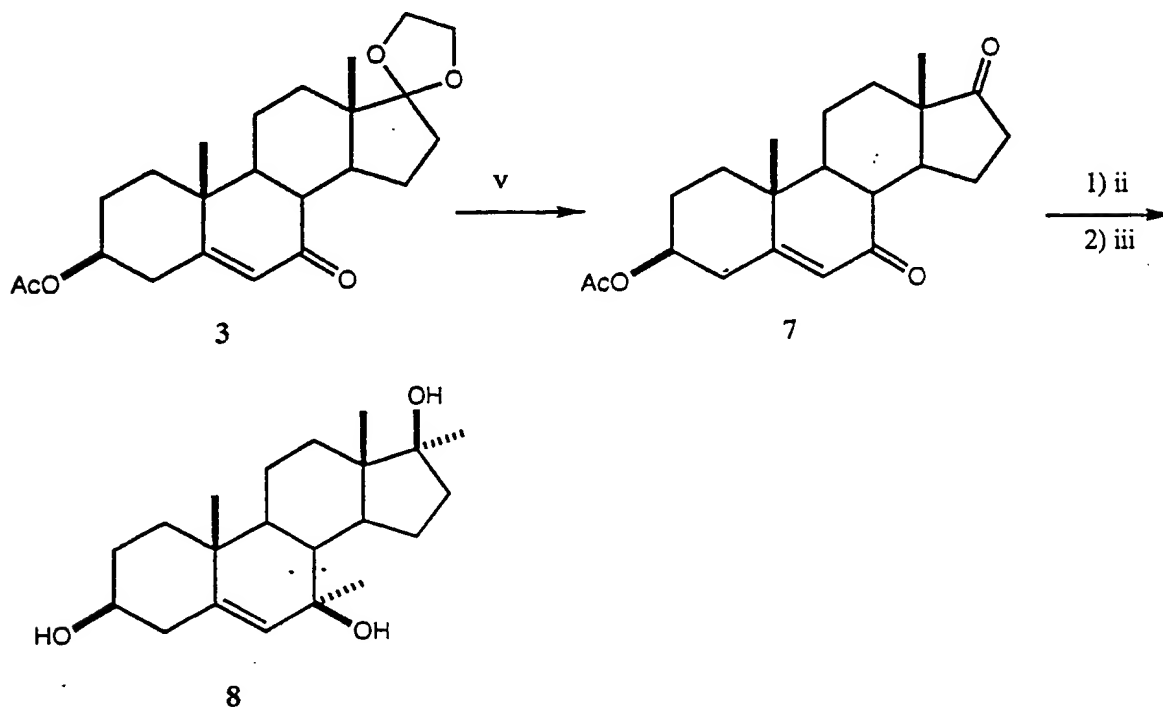
Synthesis of 17 $\alpha$ -Methyl-3 $\beta$ , 7 $\beta$ , 17 $\beta$ -Trihydroxyandrost-5-ene(6):

5        A solution of the commercially available 3 $\beta$ -acetyloxyandrost-5-en-7,17-dione-17-ethylene ketal (3 mmol) in 100 ml of dry ethanol is treated with excess solid NaBH<sub>4</sub> (379 mg, 10 mmol) with good stirring. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the  
10    reaction is >90% complete excess borohydride is destroyed by the addition of 50 ml of 1 M NH<sub>4</sub>Cl. The mixture is extracted with 150 ml of ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 4) can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of  
15    the crude product.

      A solution of 4 (1.3 mmol), from above, in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in  
20    the art. After the reaction is >90% complete the mixture is basified with NaHCO<sub>3</sub> and the methanol is evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude 5. The residue can then be purified in a manner similar to that described for 2 or carried on to the next  
25    step, depending on the purity of the crude product.

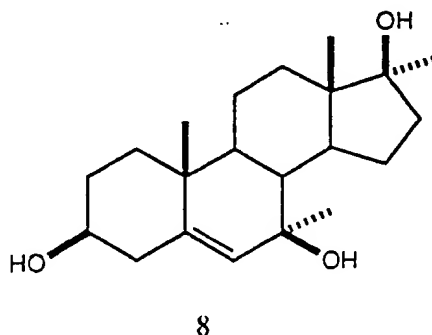
      A solution of 5 (1.3 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of CH<sub>3</sub>MgCl (Aldrich) in THF. After the addition is >90% complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and  
30    treated with 50 ml of 1 M NH<sub>4</sub>Cl in water over a 5 min. period. The resulting mixture is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and

brine, then dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give the pure title compound. The product is characterized by NMR, mass spectra, and elemental analysis.



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$   
 v)  $\text{H}_3\text{O}^+$

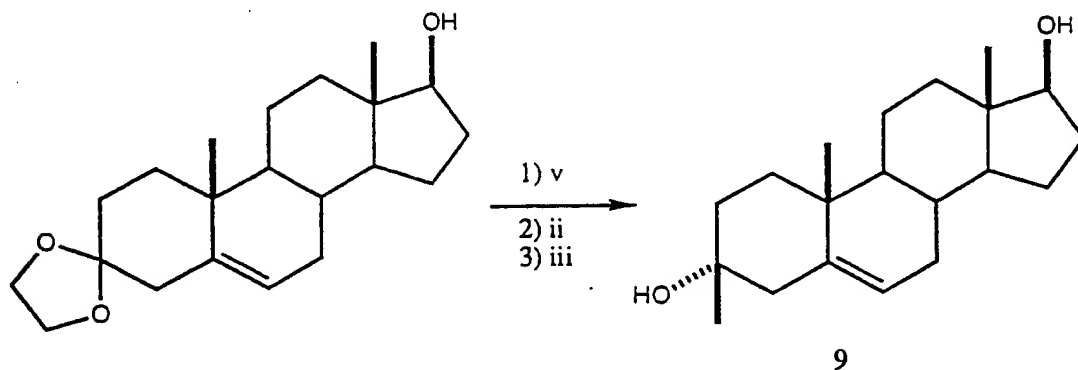
### Example 3



### Synthesis of 7α, 17α-Dimethyl-3β, 7β, 17β-Trihydroxvandrost-5-ene(8):

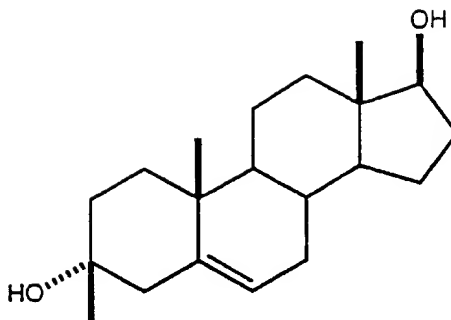
A solution of 3 (1.3 mmol), from above, in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the

- reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the mixture is basified with  $\text{NaHCO}_3$  and the methanol is evaporated under reduced pressure. The aqueous phase is then extracted
- 5 three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried ( $\text{MgSO}_4$ ) and concentrated under vacuum to give crude 7. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. This derivative (7) is also available commercially.
- 10 The resulting endione 7 (1.3 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of  $\text{CH}_3\text{MgCl}$  (Aldrich) in THF. After the addition is >90% complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and treated with 50 ml of 1 M  $\text{NH}_4\text{Cl}$  in water over a 5 min. period. The resulting mixture
- 15 is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give the pure title compound. The product is characterized by NMR, mass spectra, and elemental
- 20 analysis.



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
vi)  $\text{MeO}^-$  / MeOH, vii) Jones' reagent

## Example 4.



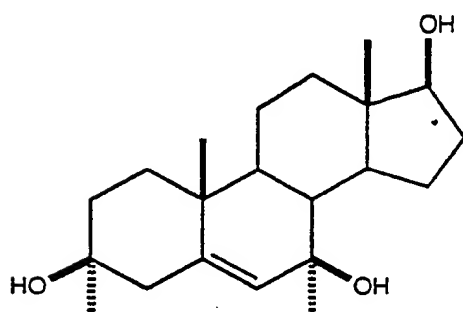
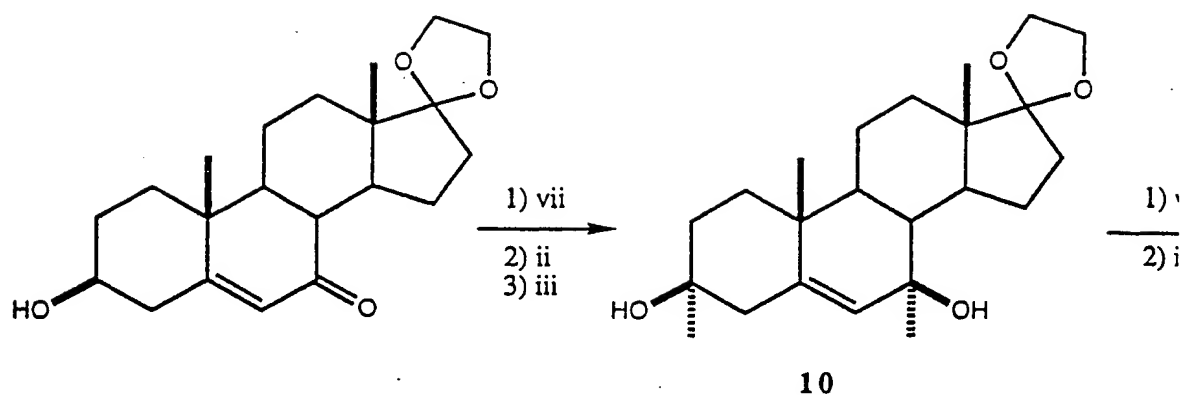
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Synthesis of 3 $\alpha$ -Methyl-3 $\beta$ , 7 $\beta$ , 17 $\beta$ -Trihydroxyandrost-5-ene(9):

- 5 A solution of the commercially available 17 $\beta$ -hydroxy-androst-5-en-3-one 3-ethylene ketal (1.3 mmol), from above, in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the
- 10 reaction is >90% complete the mixture is basified with NaHCO<sub>3</sub> and the methanol is evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude product. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step,
- 15 depending on the purity of the crude product.

- The resulting endione (1.3 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of CH<sub>3</sub>MgCl (Aldrich) in THF. After the addition is >90% complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and
- 20 treated with 50 ml of 1 M NH<sub>4</sub>Cl in water over a 5 min. period. The resulting mixture is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give the
- 25 pure title compound. The product is characterized by NMR, mass spectra, and elemental analysis.

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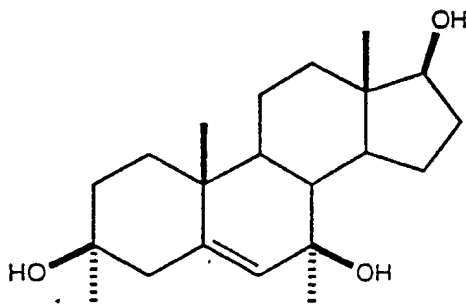


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i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
vi)  $\text{MeO}^-$  /  $\text{MeOH}$ , vii) Jone's reagent

### Example 5

5



11

Synthesis of 3 $\alpha$ , 7 $\alpha$ -Dimethyl-3 $\beta$ , 7 $\beta$ , 17 $\beta$ -Trihydroxyvandrosta-5-ene(11): Preparation of the Jone's reagent: This reagent is prepared by dissolving 26.72 g of  $\text{CrO}_3$ , in a

solution of 23 ml of conc. sulfuric which has been diluted to a final volume of 100 ml with distilled water.

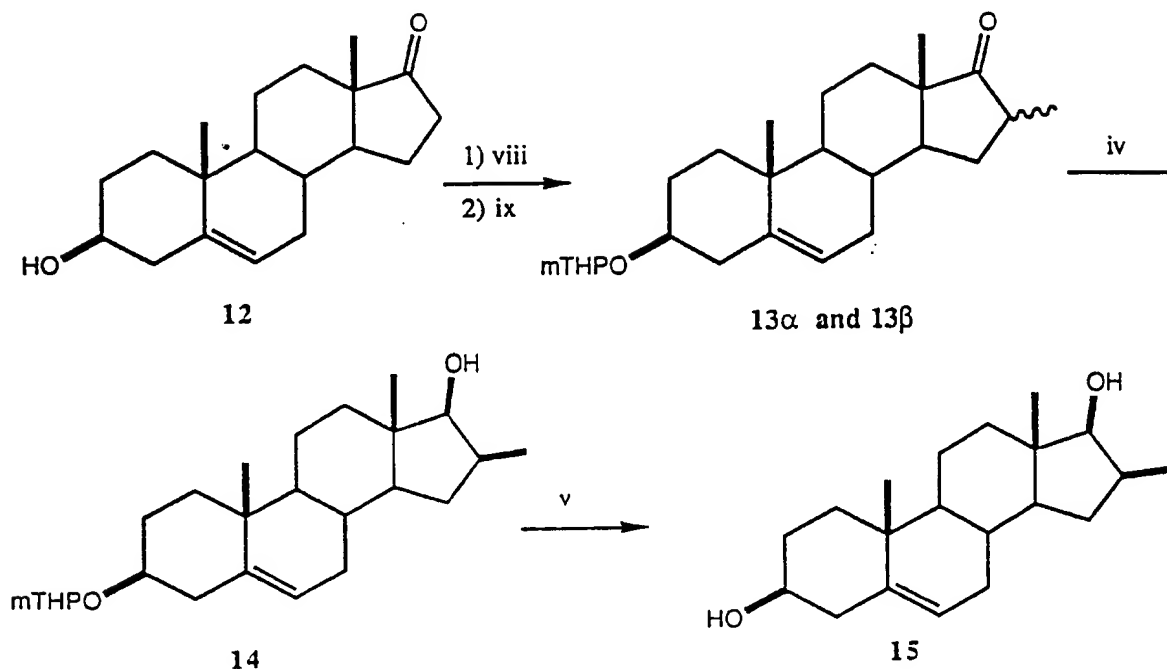
A solution of the commercially available 3 $\beta$ -hydroxy-androst-5-en-7,17-dione 17-ethylene ketal (6.0 mmol) in 25 ml of acetone is stirred in an ice-bath and treated  
5 with Jones' reagent (from above) until the orange color persists (about 3 mls). The acetone phase is separated in a separatory funnel and filtered through a plug of silica gel. The filtrate is concentrated under vacuum. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. The resulting endione (1.3 mmol) in 10 ml of THF is  
10 allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of CH<sub>3</sub>MgCl (Aldrich) in THF. After the addition is complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and treated with 50 ml of 1 M NH<sub>4</sub>Cl in water over a 5 min. period. The resulting mixture is extracted with 100 ml of ethyl acetate. The organic  
15 phase is washed with water and brine, then dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give pure diol ketal 10. The product is characterized by NMR, mass spectra, and elemental analysis.

20 A solution of 3 (1.3 mmol), from above, in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the mixture is basified with NaHCO<sub>3</sub> and  
25 the methanol is evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude 10. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

30 A solution of the product from above (3 mmol) in 100 ml of dry ethanol is treated with excess solid NaBH<sub>4</sub> (379 mg, 10 mmol) with good stirring. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete excess borohydride is  
35 destroyed by the addition of 50 ml of 1 M NH<sub>4</sub>Cl. The mixture is extracted with 150 ml of ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 11) can then be purified in a manner similar to that described for 2 to give the pure title



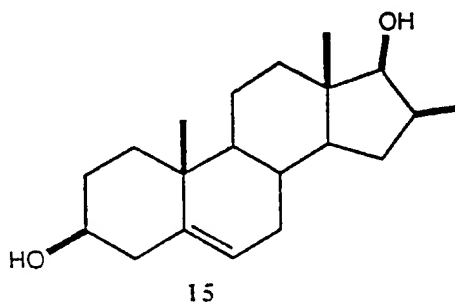
compound. The product is characterized by NMR, mass spectra, and elemental analysis.



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
 vi)  $\text{MeO}^-$ / MeOH, vii) Jone's reagent, viii) MDHP/p-TsOH/ $\text{CH}_2\text{Cl}_2$ , ix) LDA/THF,  $-40^\circ\text{C}$   
 followed by  $\text{CH}_3\text{I}$ /HMPA with warming to  $25^\circ\text{C}$ .

5

### Example 6



10

Synthesis of 16- $\beta$ -Methyl-3 $\beta$ ,17 $\beta$ -dihydroxy-androst-5-ene (15) and 16- $\alpha$ -Methyl-3 $\beta$ ,17 $\beta$ -dihydroxy-androst-5-ene (16)

A stirred ice cooled solution of the commercially available 3 $\beta$ -hydroxy-androst-5-en-17-one (20 mmol) in 20 ml dichloromethane is treated with p-

toluenesulfonic acid monohydrate (8.0 mg, 0.04 mmol) and 5,6-dihydro-4-methoxy-2H-pyran (2.6 g, 230 mmol). The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the reaction mixture is added with shaking to a mixture of 50 ml water and 75 ml ethyl acetate. The organic phase is washed three times with 50 ml 5% NaHCO<sub>3</sub>, water, and brine then dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

A solution of diisopropylamine (2.2 ml, 15.6 mmol) in 50 ml of anhydrous THF is stirred in an ice-bath under an atmosphere of dry nitrogen. This solution is treated with n-butyllithium (10.6 ml of a 1.6 M soln. in hexanes, 17 mmol). The resulting solution of lithium diisopropylamide is cooled in a -40° C bath and stirred for 0.5 h. This solution is then treated with the product from above (14.2 mmol) in 20 ml of THF by the dropwise addition over 10 min. Hexamethylphosphorous triamide (30 ml in 30 ml THF) is then added followed by iodomethane (1.99 g, 14.2 mmol) in 10 ml THF. The reaction mixture is allowed to warm to room temperature and stir for 1.5 h. The reaction mixture is then treated with 50 ml of 1 M NH<sub>4</sub>Cl in water over a 5 min. period. The resulting mixture is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (13 $\alpha$  and 13 $\beta$ , i.e. crude 13 containing the 16- $\alpha$ - and 16- $\beta$ -methyl diastereomers) can then be purified in a manner similar to that described for 2 to isolate pure 13 $\beta$ . [The 13 $\alpha$  obtained from the mixture is used to prepare 16 (see the next example)]. The residue can be purified by recrystallization (for example from ether/hexane mixtures) in combination with silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give the pure title compound. The product is characterized by NMR, mass spectra, and elemental analysis.

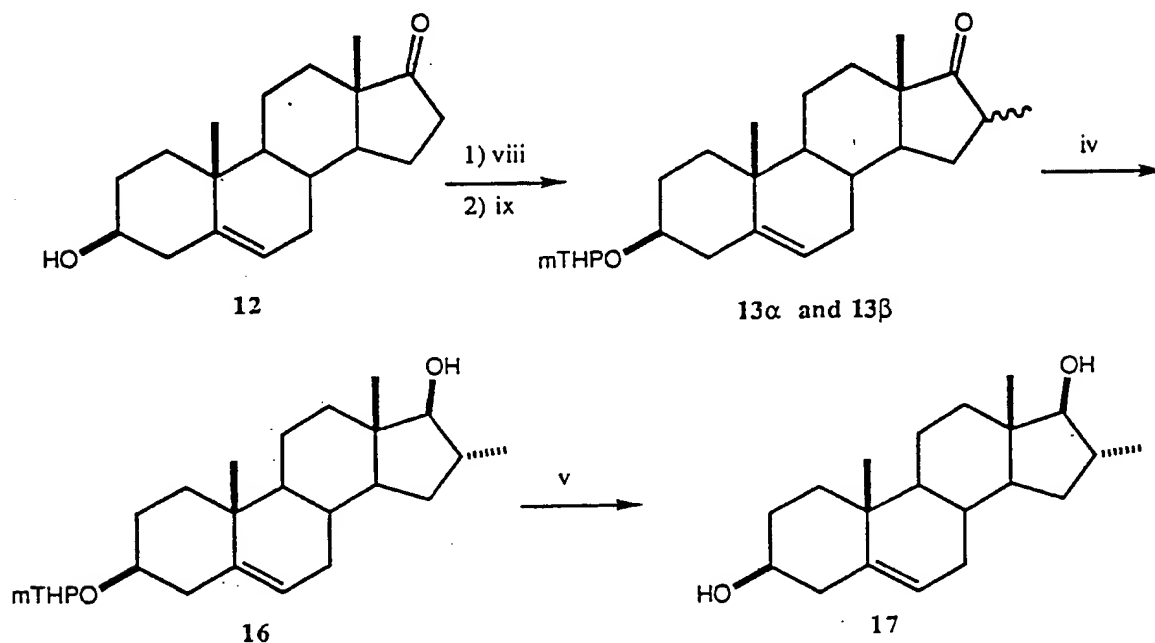
A solution of the 13 $\beta$  (3 mmol) in 100 ml of dry ethanol is treated with excess solid NaBH<sub>4</sub> (379 mg, 10 mmol) with good stirring. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete excess borohydride is destroyed by the addition of 50 ml of 1 M NH<sub>4</sub>Cl. The mixture is extracted with 150 ml of ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 14) can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

A solution of 14 (1.3 mmol), in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is

**SUBSTITUTE SHEET (RULE 26)**

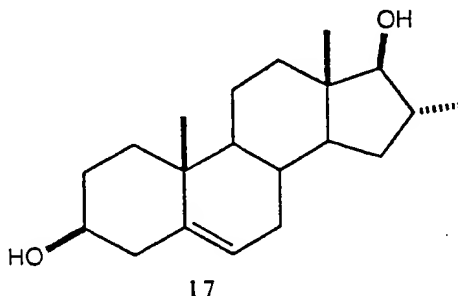
>90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the mixture is basified with  $\text{NaHCO}_3$  and the methanol is evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried ( $\text{MgSO}_4$ ) and concentrated under vacuum to give crude 15. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. The product is characterized by NMR, mass spectra, and elemental analysis.

10



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
 vi)  $\text{MeO}^-$  /  $\text{MeOH}$ , vii) Jones' reagent, viii) MDHP/ $p$ -TsOH/ $\text{CH}_2\text{Cl}_2$ , ix) LDA/THF,  $-40^\circ\text{C}$   
 followed by  $\text{CH}_3\text{I}$ /HMPA with warming to  $25^\circ\text{C}$ .

### Example 7

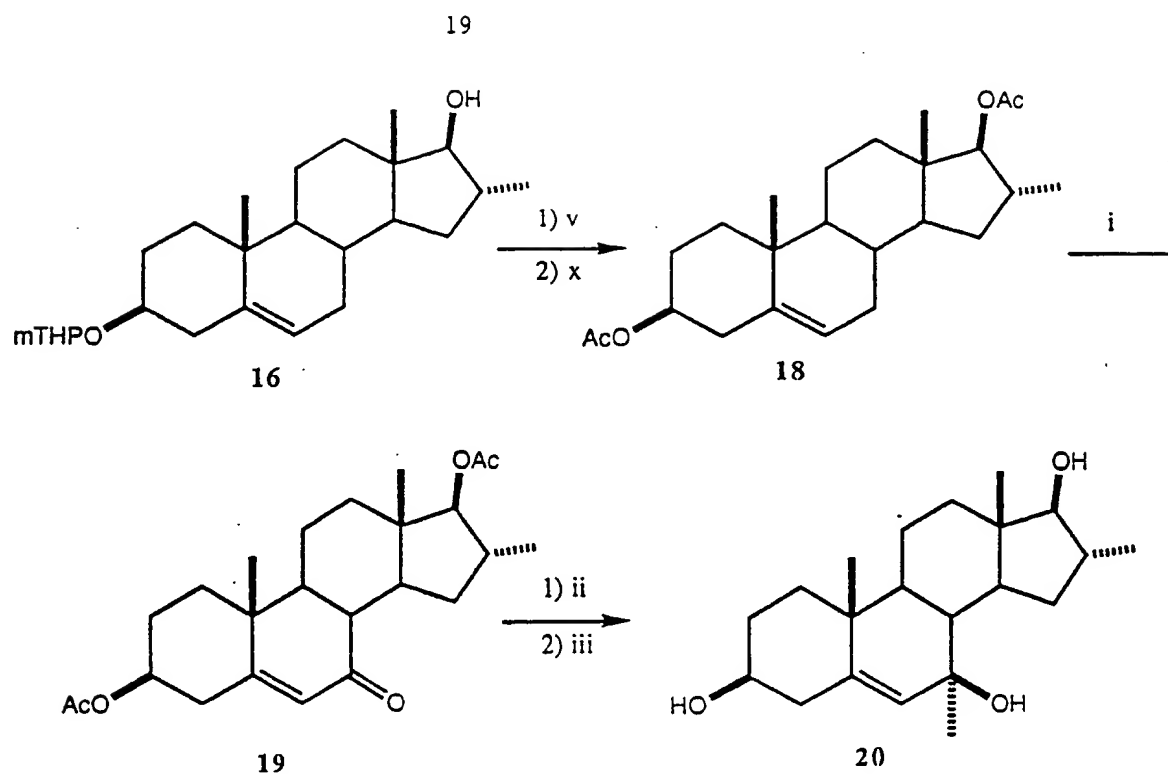


15

Synthesis of 16- $\alpha$ -Methyl-3 $\beta$ ,17 $\beta$ -dihydroxy-androst-5-ene (17)

A solution of the 13 $\alpha$  (3 mmol) in 100 ml of dry ethanol is treated with excess solid NaBH<sub>4</sub> (379 mg, 10 mmol) with good stirring. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete excess borohydride is destroyed by the addition of 50 ml of 1 M NH<sub>4</sub>Cl. The mixture is extracted with 150 ml of ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 16) can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

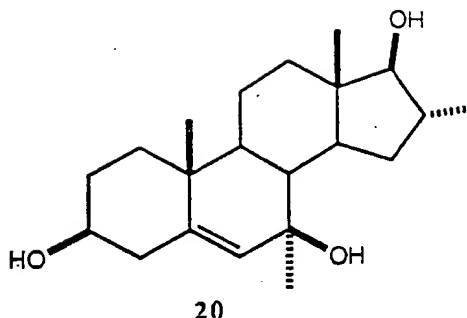
A solution of the product from above (1.3 mmol) in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the mixture is basified with NaHCO<sub>3</sub> and the methanol is evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude 17. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. The product is characterized by NMR, mass spectra, and elemental analysis.



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
 vi)  $\text{MeO}^-$ /  $\text{MeOH}$ , vii) Jones' reagent, viii) MDHP/ $p$ -TsOH/ $\text{CH}_2\text{Cl}_2$ , ix) LDA/THF,  $-40^\circ\text{C}$   
 followed by  $\text{CH}_3\text{I}$ /HMPA with warming to  $25^\circ\text{C}$ , x)  $\text{Ac}_2\text{O}$ /DMAP

20

## Example 8.

Synthesis of 16- $\alpha$ -Methyl-3 $\beta$ ,17 $\beta$ -dihydroxy-androst-5-ene (20)

- 5        A solution of 16 (3 mmol) in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete, the mixture is basified with NaHCO<sub>3</sub> and the methanol is
- 10    evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude product. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. The product is characterized by NMR,
- 15    mass spectra, and elemental analysis.

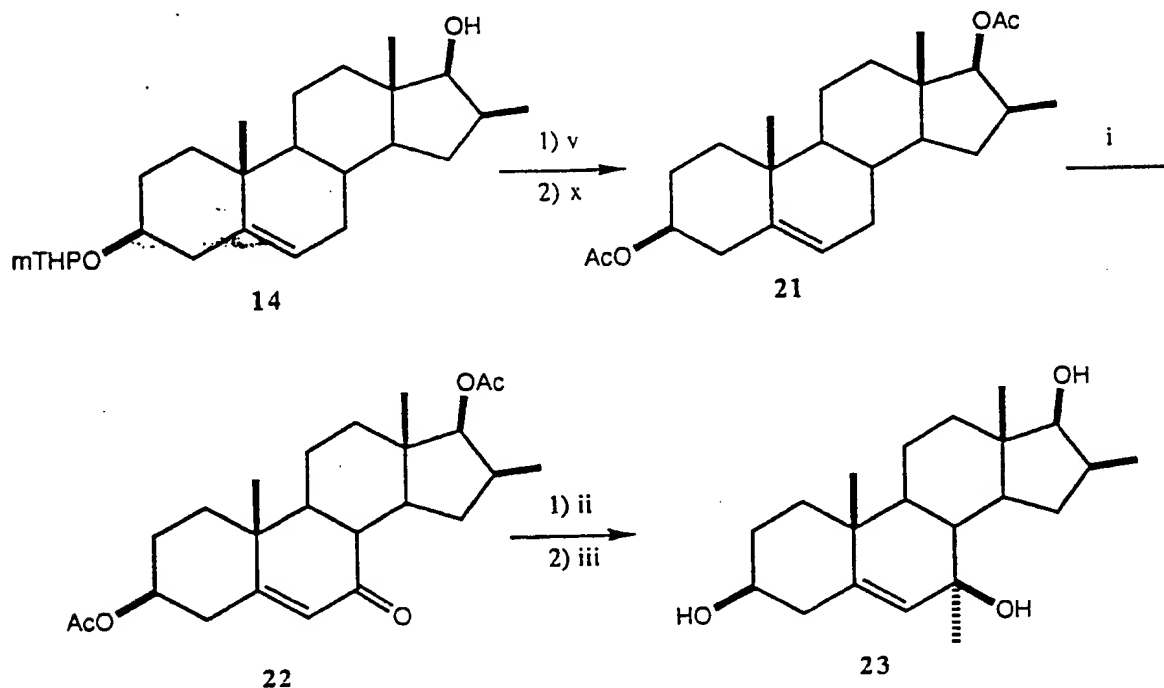
- A stirred ice cooled solution of the product from above (2 mmol) is then dissolved in 10 ml of dry pyridine and treated with 1 ml of acetic anhydride with stirring. Catalytic amounts of 4-dimethylaminopyridine can be added to increase the rate of acetylation, which is desirable in some cases. The course of the reaction can be
- 20    monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is complete 5 ml of water is added and the reaction mixture is stirred for 30 min. This mixture is then concentrated under vacuum and the residue added with shaking to a mixture of 50 ml water and 75 ml ethyl acetate. The organic phase is washed three times with 50 ml 1 M HCl, water, brine then dried
- 25    (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 18) can then be purified in a manner similar to that described for 2 or carried on to the next step depending on the purity of the crude product.

- To a solution of 18 (10 mmol) in 40 ml of glacial acetic acid is added (dropwise) a solution of CrO<sub>3</sub> (3.0 g, 30 mmol) in a 4 ml of 1:1 water:glacial acetic acid, while maintaining the temperature at 55° C for 4 h. This mixture is treated with
- 30    methanol to decompose any unreacted CrO<sub>3</sub>. The mixture is extracted with 150 ml of

ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue (crude 19) can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

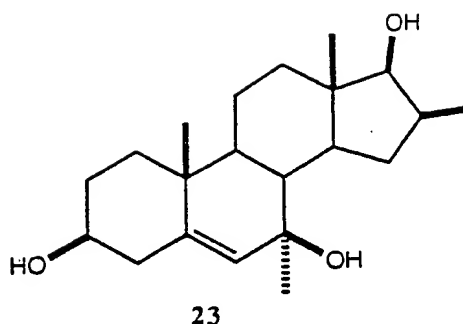
- 5 The resulting endione 19 (1.3 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of  $\text{CH}_3\text{MgCl}$  (Aldrich) in THF. After the addition is complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and treated with 50 ml of 1 M  $\text{NH}_4\text{Cl}$  in water over a 5 min. period. The resulting mixture
- 10 is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give pure
20. The product is characterized by NMR, mass spectra, and elemental analysis.

15



i)  $\text{CrO}_3$ / water/ acetic acid, ii)  $\text{CH}_3\text{MgCl}$ / THF, iii)  $\text{NH}_4\text{Cl}$ / water, iv)  $\text{NaBH}_4$ , v)  $\text{H}_3\text{O}^+$   
vi)  $\text{MeO}^-$  /  $\text{MeOH}$ , vii) Jones' reagent, viii) MDHP/ $p$ -TsOH/ $\text{CH}_2\text{Cl}_2$ , ix) LDA/THF,  $-40^\circ\text{C}$   
followed by  $\text{CH}_3\text{I}$ /HMPA with warming to  $25^\circ\text{C}$ , x)  $\text{Ac}_2\text{O}$ /DMAP

## Example 9.

Synthesis of 16-β-Methyl-3β,17β-dihydroxy-androst-5-ene (23)

5 A solution of 14 (3 mmol) in 5 ml methanol is treated with 5 ml of 10% aqueous HCl and allowed to stir. This mixture is allowed to stir until the reaction is >90% complete. The course of the reaction can be monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is >90% complete the mixture is basified with NaHCO<sub>3</sub> and the methanol is

10 evaporated under reduced pressure. The aqueous phase is then extracted three times with ethyl acetate. The combined organic phase is then washed with water and brine, dried (MgSO<sub>4</sub>) and concentrated under vacuum to give crude product. The residue can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product. The product is characterized by NMR,

15 mass spectra, and elemental analysis.

A stirred ice cooled solution of the product from above (2 mmol) is then dissolved in 10 ml of dry pyridine and treated with 1 ml of acetic anhydride with stirring. Catalytic amounts of 4-dimethylaminopyridine can be added to increase the rate of acetylation, which is desirable in some cases. The course of the reaction can be

20 monitored by, for example, thin layer chromatography, or other applicable analytical methods known in the art. After the reaction is complete 5 ml of water is added and the reaction mixture is stirred for 30 min. This mixture is then concentrated under vacuum and the residue added with shaking to a mixture of 50 ml water and 75 ml ethyl acetate. The organic phase is washed three times with 50 ml 1 M HCl, water, brine then dried

25 (MgSO<sub>4</sub>) and concentrated under vacuum. The residue (crude 21) can then be purified in a manner similar to that described for 2 or carried on to the next step depending on the purity of the crude product.

To a solution of 21 (10 mmol) in 40 ml of glacial acetic acid is added (dropwise) a solution of CrO<sub>3</sub> (3.0 g, 30 mmol) in a 4 ml of 1:1 water:glacial acetic acid, while maintaining the temperature at 55° C for 4 h. This mixture is treated with

30 methanol to decompose any unreacted CrO<sub>3</sub>. The mixture is extracted with 150 ml of



ethyl acetate, the organic phase is washed with water, and then brine. The organic phase is dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue (crude 22) can then be purified in a manner similar to that described for 2 or carried on to the next step, depending on the purity of the crude product.

- 5       The resulting endione 22 (1.3 mmol) in 10 ml of THF is allowed to stir in an ice-bath. This solution is treated with 3 ml of a 3 M solution of  $\text{CH}_3\text{MgCl}$  (Aldrich) in THF. After the addition is complete (15 min.) the solution is allowed to warm to room temperature and stir for 1 h. The reaction mixture is then cooled in an ice-bath and treated with 50 ml of 1 M  $\text{NH}_4\text{Cl}$  in water over a 5 min. period. The resulting mixture
- 10 is extracted with 100 ml of ethyl acetate. The organic phase is washed with water and brine, then dried ( $\text{MgSO}_4$ ) and concentrated under vacuum. The residue can be purified by recrystallization (for example from ether/hexane mixtures) or by silica gel chromatography (using, for example, ether/hexane mixtures as the eluent) to give pure
20. The product is characterized by NMR, mass spectra, and elemental analysis.

15

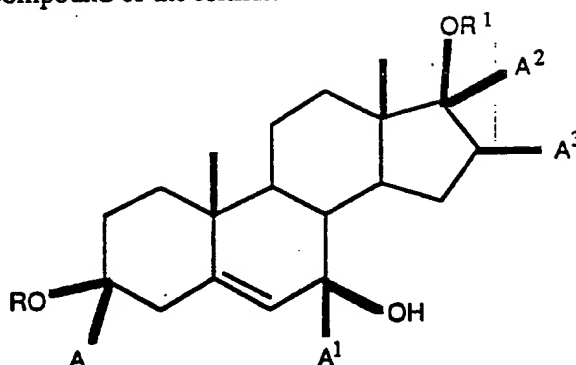
- The subject compositions find wide application for prophylaxis and therapy in a wide variety of diseases, providing for improved properties as to specificity, efficacy and safety in relation to DHEA. In addition, the subject compositions provide for new drugs which may substitute for older drugs, where a narrower range of activity is
- 20 desired.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

- 25 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A compound of the formula:



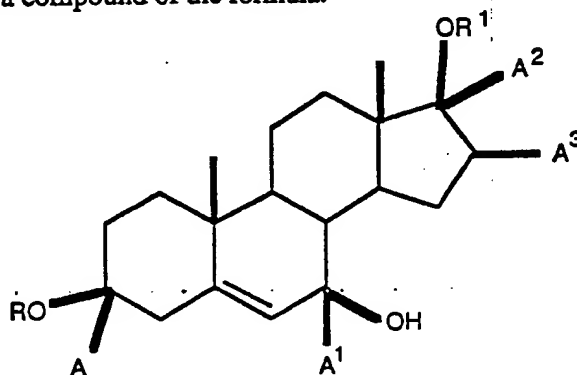
wherein:

- 5        A is hydrogen or methyl, wherein not more than 2 A groups are methyl;  
        R and R<sup>1</sup> are the same or different and are hydrogen, alkyl of from 1 to 6  
        carbon atoms, or physiologically acceptable acyl of not more than 12 carbon atoms.
2.        A compound according to Claim 1, wherein said compound is  
 10    3, 7-dimethyl.
3.        A compound according to Claim 1, wherein said compound is  
        7, 17-dimethyl.
- 15        4.        A compound according to Claim 1, wherein said compound is  
        7, 16-dimethyl.
5.        A compound according to Claim 1, wherein said compound is  
        7- $\alpha$ -methyl.
- 20        6.        A compound according to Claim 1, wherein said compound is  
        3- $\alpha$ -methyl.

7. A compound according to Claim 1, wherein R and R<sup>1</sup> are hydrogen.

8. In a method for immunizing an animal with an immunogen, the improvement which comprises:

5 administering with said immunogen in an effective amount to modulate the immune response, a compound of the formula:



wherein:

A is hydrogen or methyl, wherein not more than 2 A groups are methyl;

10 R and R<sup>1</sup> are the same or different and are hydrogen, alkyl of from 1 to 6 carbon atoms, or physiologically acceptable acyl of not more than 12 carbon atoms.

9. A method according to Claim 8, comprising the further steps of:

isolating and immortalizing lymphocytes from said animal to provide

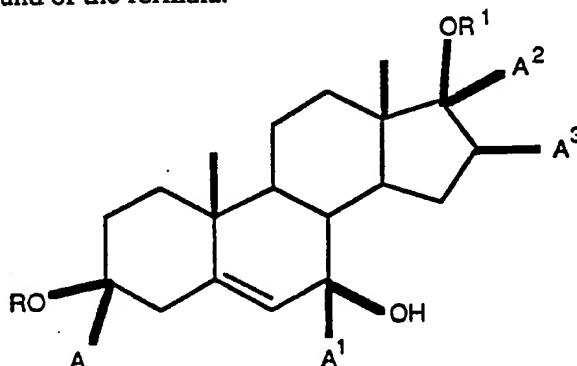
15 immortalized lymphocytes producing antibodies;

cloning and screening said immortalized lymphocytes for antibodies binding to said immunogen.

10. A method according to Claim 8, wherein R and R<sup>1</sup> are hydrogen.

11. A method for modulating the immune response of an animal, said method comprising:

administering to said mammal in an effective amount to modulate the immune response, a compound of the formula:



5

wherein:

A is hydrogen or methyl, wherein not more than 2 A groups are methyl;

R and R<sup>1</sup> are the same or different and are hydrogen, alkyl of from 1 to 6 carbon atoms, or physiologically acceptable acyl of not more than 12 carbon atoms.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/11655

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C07J1/00 A61K31/56 A61K31/565 //C07J21/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C07J A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 03925 (HUMANETICS CORPORATION) 19 March 1992 cited in the application see examples 4,5 ---	1,7,8,11
X	US,A,3 654 320 (D. AYER ET AL) 4 April 1972 see column 2 ---	1,7
P,X	THE JOURNAL OF IMMUNOLOGY, vol.153, no.4, 15 August 1994, US pages 1544 - 1552 D. PADGETT AND R. LORIA 'In vitro potentiation of lymphocyte activation by dehydroepiandrosterone, androstenediol, and androstenediol.' see the whole document --- -/--	1,7-11

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
- 'E' earlier document but published on or after the international filing date
- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
- 'P' document published prior to the international filing date but later than the priority date claimed

- 'T' later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- 'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- 'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- '&' document member of the same patent family

Date of the actual completion of the international search

5 January 1995

Date of mailing of the international search report

18.01.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
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Authorized officer

Moreno, C

## INTERNATIONAL SEARCH REPORT

Internat

Application No

PCT/US 94/11655

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US,A,5 277 907 (R. LORIA) 11 January 1994 see column 1 - column 4 ---	1,7,8,11
P,X	WO,A,94 08588 (CONSERVATOIRE NATIONAL DES ARTS ET METIERS) 28 April 1994 see claims; examples -----	1,7,8,11

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/ 11655

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 8-11  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 8 to 11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Application No  
PCT/US 94/11655

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9203925	19-03-92	AU-B- 647528	24-03-94
		AU-A- 8541891	30-03-92
		CA-A- 2090759	01-03-92
		EP-A- 0547151	23-06-93
		JP-T- 6508345	22-09-94
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